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DURATION OF AN AFTER-EFFECT IN PLANARIANS FOLLOWING A REVERSED HORIZONTAL MAGNETIC VECTOR[1]

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Responsiveness of the living system to very weak magnetic fields is well established. Studies have involved organisms ranging from the unicellular *Paramecium* (Brown, 1962), through *Volvox* (Palmer, 1963), *Dugesia* (Brown, 1962), *Nassarius* (Brown, Brett, Bennett and Barnwell, 1960; Brown, Webb and Brett, 1960; Brown, Bennett and Webb, 1960; Brown, Webb and Barnwell, 1964), *Drosophila* (Picton, 1964), and Cockchafers (Schneider, 1963), to birds (Eldarov and Kholodov, 1964). The nature and strength of the response have been found to vary as functions of such factors as orientation of the experimental horizontal magnetic vector, of geographic orientation of the organisms, and of phase angles of the natural solar and lunar cycles.

It was recently demonstrated, furthermore, that the mud-snail, *Nassarius,* is able to distinguish strength differences of the horizontal magnetic vector at least within the range, 0.05 to 10.0 gauss, displaying a maximum in capacity to respond to an experimentally reversed horizontal magnetic vector when the directional change is effected with minimal simultaneous change in vector strength (Brown, Barnwell and Webb, 1964). Following subjection to horizontal vector fields differing from the earth's 0.17-gauss local one, the snails, upon return to the natural field, continued to display an influence of the experimentally imposed field. The effect lasted for at least three to five minutes. An after-effect of experimental magnetic fields, with strikingly similar characteristics, was discovered also for the planarian, *Dugesia* (Brown, 1965). An after-effect has also been reported for birds (Eldarov and Kholodov, 1964).

The present study was directed toward learning how long the after-effect, which results from an experimentally reversed magnetic field, persists beyond the previously demonstrated short period, and to learn something concerning the characteristics of its decay. More knowledge concerning these characteristics is of

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fundamental importance for the ultimate formulation of a sound hypothesis for the nature of the magnetoreceptive mechanism.

Materials, Methods and Experiments

Planarians, *Dugesia dorotocephala*, were observed as they moved initially northward over a polar-coordinate grid in an asymmetrical three-light field (Brown, 1962) during about a seven-month period from October 8, 1963, through April 30, 1964. The experimental conditions were designed to reveal the duration of the after-effect following response to a reversed magnetic field. Experimental series were usually run in pairs. Each of the two observers involved in a double series recorded planarian paths as follows. Five worms were placed in a 9-cm. Petri dish of water inside an orientation chamber where they remained for the duration of a 50-minute series. First, two 5-minute samples of worm-paths, to serve as controls, were recorded under the natural ambient environmental conditions. Thereupon, an 18-cm., cylindrical, alnico bar magnet was placed horizontally, centered directly beneath the point of initiation of the worm path, oriented to oppose the direction of the earth's horizontal vector, and located at such a distance below the orienting worms as to produce at the level of the worms a reversed horizontal field of the desired strength. Immediately thereafter the mean rate of turning of all the worms orienting during the first, the second, and the third five-minute interval was determined separately. The experimental magnetic field was then removed and the mean path for all the orienting worms was determined for each of the subsequent five-minute intervals during a 25-minute period.

One observer dealt with a reversed magnetic field of 4.0 gauss; another observer used a reversed field of 0.05 gauss. During the course of the 7-month experiment a total of five different observers participated and all observers dealt with both magnetic field strengths.

The observations were made at various hours of the day between 9 AM and 5 PM. About 60% were made during the morning hours. The total number of series run for each of the two magnetic field strengths for each of the seven calendar months was as follows:

	4.0 gauss	0.05 gauss
October	57	61
November	78	79
December	47	45
January	67	64
February	43	48
March	28	26
April	37	37
Total	357	360

It was learned later that the actual average number of worm-paths that was obtained in a 5-minute interval was about 14. The number varied from day to day depending upon the degree of activity of the animals. It is evident from the foregoing that during the 7-month period of the investigation there were about 10,000 initial control paths, about 15,000 paths during the 15-minute period of the applied fields, and about 25,000 paths during the 25 minutes following removal of the experimental fields.

The data were reduced in the following manner. For each of the single 50-minute series, the mean paths of the worms during each of the three 5-minute intervals while in the experimental field and during each of the five 5-minute intervals after removal of the field were expressed as differences from the average path determined for the initial two control samples in the same series. Mean values obtained in this manner for the "responses" to each of the two field strengths were then determined for all the data for successive 5-calendar-day periods. In addition, the mean was computed for each of the eight temporally corresponding differences between responses to the two experimental magnetic field strengths. Analyses of this report were based upon the eight values for each of the 5-day periods for the two experimental field strengths. This yielded a total of 656 individual data, or 328 differences.

It was quite conceivable that the worms would adapt to each of the two altered experimental fields in the course of time, and that the initial kind of turning behavior might gradually be restored despite continued presence of the experimental, reversed magnetic fields. To establish or eliminate this possibility, an additional experiment was performed. This second experiment was conducted between October 27 and December 22, 1964. It was designed just like the initial one except that the magnets were left in place during the whole 40 minutes instead of being removed after 15 minutes. It differed in addition, however, in the following ways; (1) Only two months were involved, overlapping in time of year, one year later, only about one-third of the period of the earlier experiment. (2) Six observers contributed to the results; four of them had not participated in the earlier observations and were given no information about either the earlier results or even the objectives of the study. (3) A larger fraction of the observations, about 65%, were made during afternoon hours. A total of 30 series was run at 0.05 gauss and 32 series at 4.0 gauss, distributed over the whole two-month period.

The data of this second experiment were treated like the earlier data in that responses were determined separately for each of the 5-minute periods by obtaining the path differences from those of the initial controls. The differences between the mean responses to each of the two field strengths for the corresponding consecutive time intervals were computed. The mean response to each strength for the first 15 minutes, the last 15 minutes and the intervening 10 minutes was calculated, together with standard errors of their means.

Results

A. Removing magnets after 15 minutes

It is evident that if there were no influence of either of the two imposed reversed magnetic field strengths, no significant difference between the mean paths of the worms for the corresponding, successive pairs of 5-minute samples would be expected between the results for the two fields. If responses did occur, absence of significant difference between the two would indicate that exactly the same response resulted for the two strengths. Other than in magnetic-strength difference, the worms for the two kinds of series had been treated in precisely the same general manner by the same observers over a sufficiently long period to have reduced to insignificance the differences between the two means due to random sampling.

This method would also compensate for any systematic change that might tend to occur in the orientation of the worms over the course of the 50-minute observation period while in the orientation chamber. Such a systematic change could conceivably arise from a gradual photic adaptation, the repeated mechanical stimulation, or simply fatigue of the five worms. The procedure employed would

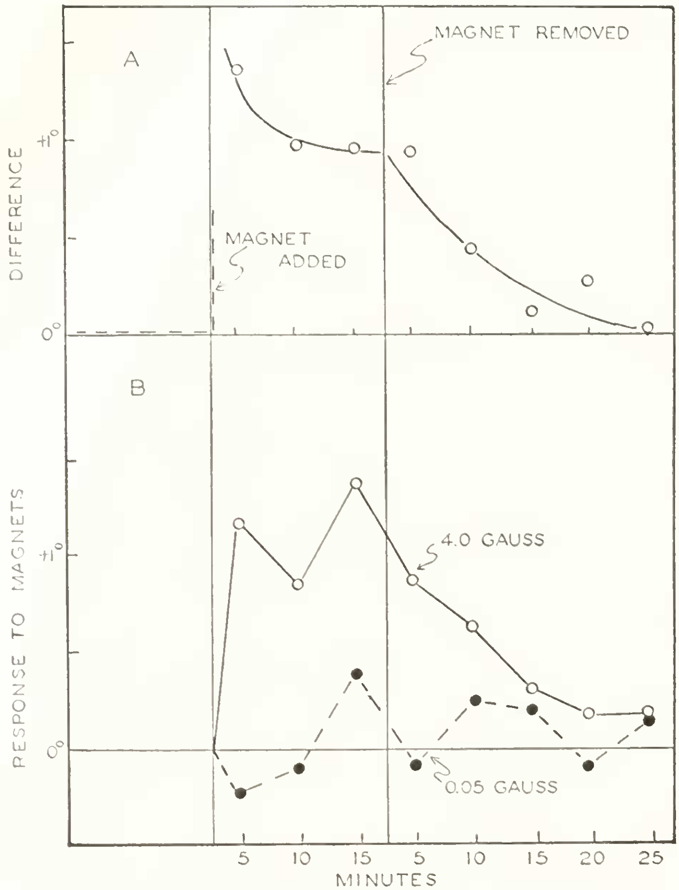

FIGURE 1. A, the response to a reversed 4-gauss horizontal magnetic field, expressed as difference from response to a reversed 0.05-gauss one, during three consecutive 5-minute intervals after application of the experimental fields, and during five 5-minute intervals following field removal. B, the responses to the two strengths of magnetic field, expressed as differences from initial controls, during and following the experimental field reversals.

be expected to eliminate, therefore, all changes which were independent of the specific differences between the effects of the two experimental magnetic treatments.

A previous study (Brown, 1965) had established two facts: (1) north-directed worms in a 4.0-gauss reversed field turned more strongly clockwise than the same worms when they were subjected to a 0.05-gauss reversed field, and (2) during the initial 5-minute period following removal of the reversed field the worms that had been exposed to 4- to 6-gauss fields turned more strongly clockwise than the same worms subsequent to removal of 0.05- to 0.1-gauss fields.

In Figure 1 A, the mean path of the worms for each 5-minute interval in the 4.0-gauss series is plotted as the difference from the mean path for the corresponding interval in the 0.05-gauss series. Confirming the results of the previous study (Brown, 1965), worms in the 4.0-gauss field turned clockwise relative to worms in the 0.05-gauss one. There is suggestively present an initial overshoot and a subsequent tendency toward stabilization at a slightly decreased difference during the 15-minute period of exposure. Upon removal of the experimental, reversed fields, an after-effect persists with the same sign and of about the same magnitude during the first 5-minute interval. This is followed by a gradual decay of this after-effect with the difference between the after-effects from the two imposed experimental magnetic fields disappearing completely by the end of about 25 minutes.

It is seen from Figure 1 B, in which the mean responses of the worms to each of the two experimental field strengths, together with the post-field changes, are shown separately, that the results illustrated in Figure 1 A appear to come chiefly from a mean clockwise response to the 4.0-gauss field relative to essentially no mean response to the 0.05-gauss field.

B. Leaving magnets in place for 40 minutes

In Figure 2 A are seen the differences between responses of worms while in the reversed fields of 4.0 and 0.05 gauss for each of the eight consecutive 5-minute samples. In Figure 2 B the response to each of the field-strengths is shown separately. Although this figure is entirely comparable to Figure 1, it will be noted that the scale has been altered by a factor of 4. Both the responses of the worms to each field strength and the difference between the responses were much greater than for the earlier experimental series. In addition, a clear mean response was evident not only for the 4.0-gauss field (clockwise turning) but for the 0.05-gauss one (counterclockwise turning) as well.

One fact was immediately apparent from the results of this experiment, namely that there was no overall reduction in the influence of the experimental fields on the worms during the 40 minutes of exposure to these fields. First, considering grossly the differences between responses to the two field strengths, the difference was found for the first 15 minutes in the fields to be $3.87 \pm 0.921°$ ($N = 186$) and for the last 15 minutes of the 40-minute exposure to be $3.97 \pm 0.822°$ ($N = 186$.) Examining each field strength separately, for the 0.05-gauss field the first and last 15-minute groups of responses were $-2.78 \pm 0.605°$ ($N = 90$) and $-2.79 \pm 0.582°$ ($N = 90$), respectively. For the 4.0-gauss field, the comparable values were $+1.09 \pm 0.695°$ ($N = 96$) and $+1.18 \pm 0.581°$ ($N = 96$). The two mid-series values were $+1.44 \pm 0.559°$ ($N = 62$) for the 4.0 gauss and $-1.61 \pm 0.678°$ ($N = 60$) for 0.05-gauss.

It had been suggested, from examination of Figure 1, that there was an initial over-shoot in the response to the reversed fields with a stabilization at a slightly lower level prior to the removal of the experimental fields at the end of 15 minutes. A very similar behavior occurred again in this second experiment and, indeed, a partial apparent acclimation suggestively continued for about 20 minutes. Also as in the first series this behavior seemed to result principally from an alteration in response to the weaker, 0.05-gauss, field and only to a lesser extent to the 4.0-gauss one. But equally suggestive was an apparent complete loss of this partially "acclimated" state by the end of the 40-minute period of observation.

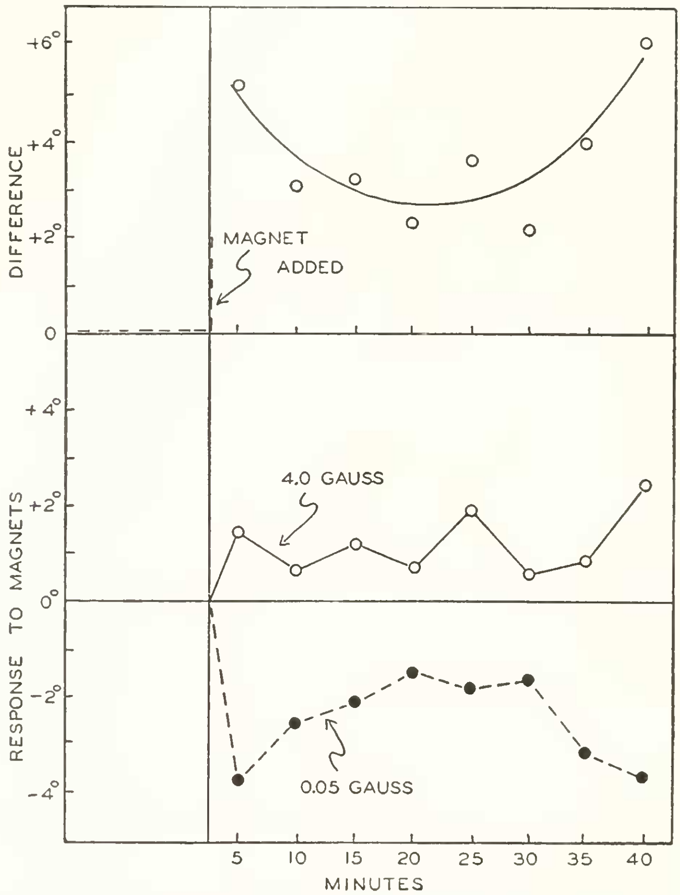

FIGURE 2. A, the response to a reversed 4-gauss horizontal magnetic field, expressed as difference from response to a reversed 0.05-gauss one, during eight consecutive 5-minute intervals during application of the experimental fields. B, the responses to the two strengths of magnetic field, expressed as differences from initial controls, while being subjected to experimental field reversals.

In view of the unexplained difference between the first and second experiment with respect to (a) the amplitude of responses to the two reversed fields and differences between the two responses, and especially (b) the difference between the series relative to the apparent clear response to the 0.05-gauss field in the second, but not in the first experiment, an additional analysis of the data was made. This consisted of computing separately the mean responses to each of the two reversed field strengths while in the field, for each of the 9 months for which data had been obtained. The results are illustrated in Figure 3.

It is apparent from Figure 3 that the responses to the two reversed horizontal-field strengths varied through a relatively large range, even from clockwise to counterclockwise turning. The only common feature was that for every one of the nine months the turning in response to the stronger field was clockwise relative to the response to the weaker one. This was true whether both responses were clockwise, or counterclockwise, or one was clockwise and the other counterclockwise. The apparent absence of a measurable response to the 0.05-gauss field, noted in Figure 1, can now be seen to have resulted from a clockwise turning

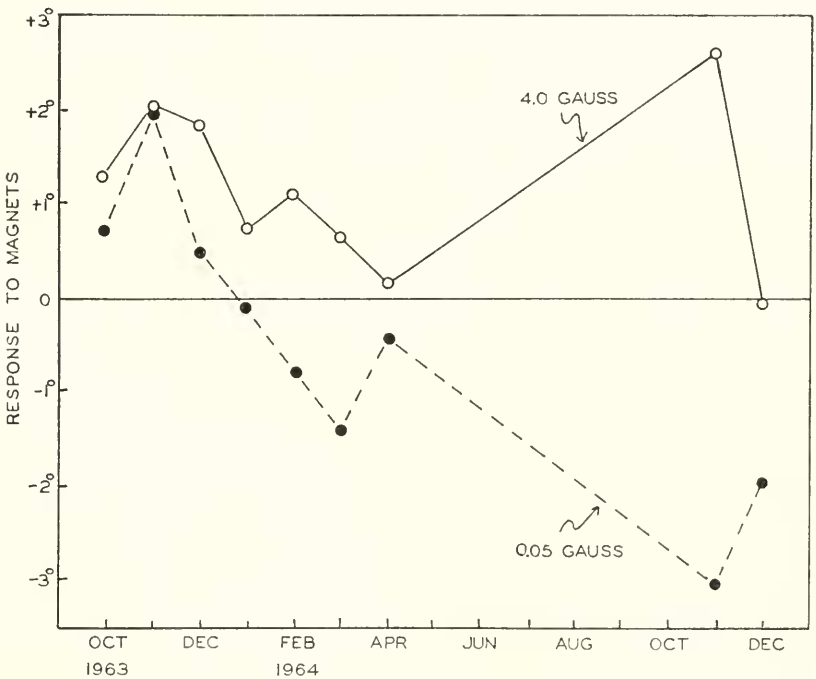

FIGURE 3. The mean responses to reversed 0.05- and 4.0-gauss horizontal magnetic vectors that were obtained for each of nine calendar months of the investigation in 1963 and 1964.

response during the first three months being balanced by an approximately equal counterclockwise turning response for the last three months. When responses to the two field strengths were in the same direction, the responsiveness tended to vary parallelly, and when in the opposite direction to vary in an opposite or mirror-image manner. Maximum in responsiveness for both field strengths came during Novembers, though for the first November responses to the two strengths possessed the same sign and for the second November they were of opposite sign. In addition to this possible annual variation in strength of the responses, a definite monthly variation in these data was discovered and will be reported later in another connection.

Discussion

These results confirm in a striking manner earlier reported ones with not only planarians (Brown, 1965) but also mud-snails (Brown, Barnwell and Webb, 1964) in demonstrating a difference between the after-effects on orientational behavior between exposures to reversals of horizontal magnetic vector fields, stronger and weaker than the ambient local one. These field reversals are, of course, reversals relative to all other contemporary ambient vector fields. At the same time, evidence is offered supporting a conclusion that following removal of the experimental field, there is a gradual, exponential decay in the altered state, requiring from 20 to 30 minutes for essentially complete loss.

In addition, this study has suggested strongly that following an abrupt reversal of the horizontal vector of the magnetic field there is an initial maximum response followed by some compensatory reaction which, in the continuing experimental field, proceeds for about 20 minutes. This seems evident in the present experiment principally for the weaker field. This compensatory alteration seems not only to cease but to be followed by a return of the planarians toward a response state of the degree present in the initial response to the abruptly altered field. In brief, there appears to occur a phenomenon which might be termed a "transient accommodation."

Despite the relatively clear demonstration of responsiveness to these weak magnetic fields when essentially simultaneous control and experimental data are compared, and analyses are based upon differences within concurrently obtained data, the drift with time in character of the response to carefully reproduced experimental conditions in the laboratory, such as is illustrated in Figure 3, emphasizes that one is dealing with a biological response within the range of responses normally effected by natural geophysical fluctuations. Other, uncontrolled environmental variables are continuing to exercise influences of the same order of magnitude as the specific ones being investigated, and within these fluctuations are monthly, and suggestively substantial annually, varying components. Variations in these uncontrolled factors are able clearly to modify in a highly significant manner even the response to our controlled experimental alterations. It is of interest to note that November extremes which have been recorded here parallel a late fall extreme value in several other, previously-reported annual variations persisting in so-called constant conditions (Brown, 1964).

One final matter should be mentioned, namely implications of this study for compass responses of animals in unvarying fields of illumination. To the extent that these compass-directional differences in orientational behavior are non-adaptive responses to the earth's geophysical vector fields and depend upon magnetism, this behavioral response would be expected to exhibit variations with time, including not only the already established monthly variations as in snails (Brown, Webb and Barnwell, 1964), but also an annual variation as previously shown for planarians (Brown, 1962, 1963). In addition, longer, secular trends and non-periodic fluctuations of substantial range might reasonably be expected. Similar variability need not be, however, a necessary consequence when adaptive orientational behavior patterns of animals depend upon an informational input from the total contemporary geophysical scene, information which is integrated and interpreted by the adaptively responding organism.

Summary

1. Planarians exhibit a significant difference between response to a reversed horizontal 0.05-gauss and a similarly reversed 4.0-gauss magnetic vector.

2. A difference between orientational behaviors in the natural field following 15-minute exposures of the worms to these two reversed field strengths persists for 20–30 minutes.

3. This after-effect decays exponentially with time.

4. Subjected to an abrupt reversal in horizontal magnetic field, an initial maximal response appears to be followed by a transient accommodation continuing for about 20 minutes. This partial accommodation is lost again completely by the end of 40 minutes. This transient accommodation appeared more evident for the weaker field during these studies.

5. It is shown that the response to the experimental magnetic field reversals varied substantially over the course of the 15-month period of the investigation, in a manner suggesting strongly the existence of relatively large influences of other uncontrolled geophysical variables, factors with biological influences of the same order of magnitude as those being investigated.

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